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## Occupational paraquat exposure of agricultural workers in large Costa Rican farms

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## Abstract

**Objective**—Paraquat is an herbicide widely used worldwide. This study determined the extent of occupational exposure to paraquat among farm workers in Costa Rica and identified determinants of occupational exposure.

**Methods**—Twenty-four hour urine samples were collected from 119 paraquat handlers and 54 non-handlers from banana, coffee and palm oil farms. Information about herbicide handling operations was also collected. The urinary paraquat levels were determined by an enzyme-linked immunosorbent assay (ELISA) with a limit of quantification (LOQ) of 2 ng/mL. Inhalable dust and airborne paraquat levels were simultaneously measured for a subset of the participants.

**Results**—Urinary paraquat measurements were non-detectable or very low when workers did not handle paraquat. For handlers, 83.3, 47.1 and 63.9% of the samples were below the LOQ on before-, during- and after-paraquat spray days, respectively. The arithmetic mean ( $\pm$ SD) of urinary paraquat level on days when workers handled paraquat was 6.3 ( $\pm$ 10.45)  $\mu$ g/24h. Paraquat exposures among handlers on spray day were significantly associated with the type of crop.

**Conclusion**—Non-handlers had negligible urinary paraquat, while detectable paraquat exposures were observed among handlers on spray day. Urinary paraquat levels were different by crop.

### Keywords

Paraquat; Herbicide; Occupational exposure; Biological monitoring

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### Introduction

Paraquat (1,1'-dimethyl-4,4' bipyridinium dichloride), a cationic bipyridylum class herbicide, is a non-selective and non-systemic herbicide that has been used widely throughout the world over the past 40 years. It is classified as moderately toxic via the oral route (EPA category II) and as slightly toxic by the dermal route (EPA category III) (US Environmental Protection Agency (EPA) 1997). Absorption through intact skin is minimal, and inhalation toxicity is not considered by EPA, as paraquat has no appreciable vapor pressure and there is no hazard from gaseous inhalation. The threshold limit value of paraquat is 0.1 mg/m<sup>3</sup> for respirable fraction and 0.5 mg/m<sup>3</sup> for total dust (American Conference of Governmental Industrial Hygienists (ACGIH) 2008). The most common formulation is an aqueous solution containing 200 g of paraquat per liter. The concentrated formulation is diluted with water before application. Paraquat is most commonly applied by spray methods using a hand-pressurized backpack. Prolonged and direct contact with the spray mist may cause oral and nasal irritation as well as possible absorption.

Determination of paraquat level in urine is a useful way to measure the extent of absorption for exposure assessment. Animal studies have shown that the absorbed paraquat is mainly excreted through the kidney (Daniel and Gage 1966), with almost 100% of the absorbed paraquat eliminated in an unchanged form in the urine within 24 h (Hawksworth et al. 1981). Paraquat levels in blood were low compared with those in urine (Tompsett 1970).

Occupational exposure to paraquat has been assessed in several observational studies. Only one study assessed the exposure using 24-h urine collection; however, the study was unable to detect any urinary paraquat (Chester et al. 1993). Biological monitoring of paraquat levels in spot urine samples collected at the end of the workday has been utilized by other researchers (Chester and Woollen 1981; Staiff et al. 1975; Swan 1969; Van Wendel de Joode et al. 1996; Wojcek et al. 1983). There was no paraquat residue detected in spot urines among paraquat applicators working in field and garden (Staiff et al. 1975). In two studies conducted with Malaysian rubber plantation workers, only 53 of 394 subjects had detectable urinary paraquat in end-of-shift spot samples (Swan 1969), and 10 out of 26 subjects had detectable urinary paraquat in end-of-shift spot samples (Chester and Woollen 1981). Two of 28 spot urine samples had detectable paraquat levels at Costa Rican banana farms (Van Wendel de Joode et al. 1996). In Florida farms for plant residue destruction (tomatoes) or weed control (citrus), 1 of 13 applicators had detectable urinary paraquat in spot samples (Wojcek et al. 1983). The low frequency of urinary paraquat detection in these studies was likely related to a high limit of detection (LOD) in urine that ranged from 10 to 50 ng/mL (Chester and Woollen 1981; Staiff et al. 1975; Swan 1969; Van Wendel de Joode et al. 1996; Wojcek et al. 1983).

Spot urine samples may not represent the overall profile of absorbed paraquat, since paraquat levels can be influenced by urine collection time and exposure profile. Although a 24-h urine sample provides a more complete profile for determining the overall absorbed paraquat, such a method requires an improved analytical method to detect very low concentrations of target compounds in the 24-h urine samples. Conventional analytical methods including gas chromatography or high performance liquid chromatography are available to monitor the paraquat from many matrices but are limited by the high cost, the labor-intensive methods and

the sample cleanup required (Blake et al. 2002; Hajslova et al. 1989). LC-MS is a promising technique but requires extensive instrumentation. Alternatively, an enzyme-linked immunosorbent assay (ELISA) can satisfy the demands for faster, cheaper and more sensitive analytical methods for use in large scale field studies (Van Emon et al. 1986), Koivunen et al. (2005) reported an ELISA method for determining paraquat ion with an LOQ of 2 ng/mL in urine samples. The LOQ in urine with the ELISA method in this study is 5- to 25-fold more sensitive than the LOD levels in urine reported in previous paraquat biological monitoring studies (Chester and Woollen 1981; Staiff et al. 1975; Swan 1969; Van Wendel de Joode et al. 1996; Wojcek et al. 1983).

The objectives of this paper are to measure the absorbed paraquat accurately by collecting 24-h urine in order to determine the extent of occupational paraquat exposure and to identify the determinants of occupational paraquat exposure among workers on large farms in Costa Rica. Airborne paraquat exposure was also measured in a subset of paraquat handlers.

## Methods

### Study population and setting

We identified 62 coffee, banana and palm oil farms that purchased paraquat from a major pesticide dealer in 2000 in Costa Rica. Of the 62 farms initially identified, a total of 16 farms were eligible and agreed to participate in the study, including five banana farms, ten coffee farms and one palm oil plantation. Eligibility criteria were current use of paraquat, more than 3 applicators, and paraquat application periods matched to our study duration. Average farm sizes were 336 hectares (ha) for the five banana farms (range 250–600 ha), 341 ha for the ten coffee farms (range 85–1,600 ha) and 11,935 ha for the palm oil farm.

Handlers were defined as farm workers who mix, load or spray paraquat. Non-handlers consisted of general plantation workers with no paraquat handling or pesticide applicators whose activities did not involve any reported paraquat exposure during the biological monitoring periods. Among 187 handlers from the 16 farms, 134 handlers were eligible. One hundred nineteen handlers (63.6%) participated and 15 handlers (8.0%) refused to participate. Fifty-three handlers were ineligible: 49 handlers (26.2%) performed other jobs, such as spraying insecticide or hand weeding on the sampling day, and four handlers (2.1%) were absent on the sampling day. Participation in this study was voluntary and informed consent form was obtained. The study protocol and procedures for informed consent were reviewed and approved by the institutional review boards of the University of California at Davis and the Costa Rican Ministry of Health. Farms were not compensated for participation, but participants were provided small non-monetary incentives. The field study period was from May to December 2001, the rainy season in Costa Rica.

### Biological monitoring

Twenty-four hour urine samples were collected on the day prior to paraquat spraying, on the spray day and on the day after spraying. The number of samples collected on the day prior to paraquat spraying and on one day after spraying was less than on the spray day. This occurred because handlers were often applying 6 days per week, and it was difficult to collect urine samples during the weekend. Participants did not handle paraquat at least 1 week before biological monitoring commenced. For the urine collection, each participant received a 4 L amber-colored container made of a mixture of polypropylene and polyethylene with a 79 mm opening. Participants were also provided with a container-carrying bag, written instructions for urine collection, a self-written time sheet and a pen. Twelve grams of boric acid ( $H_3BO_3$ ) were added to each container to prevent contamination of the urine by microorganisms. Participants were instructed to discard the first urine void on the first urine collection day, and

all subsequent voidings were then collected into the container, including the first urine void on the next morning. First and last void times were recorded on the time sheet. To reduce any possibility of contamination in urine by extraneous paraquat from their hands and clothes, the participants were asked to use extra caution while urine was collected into the container.

A short questionnaire was administered on the day after the sampling period by the field staff when the participants returned their urine samples at the workplace before beginning work. Information collected included job tasks, location of loading and mixing paraquat, paraquat spray methods, use of personal protective equipment (PPE) and participant's assessment of the completeness of the 24-h urine collection.

Upon receipt of the urine samples, total urine volume was measured and recorded in the field. After vigorously shaking the container, an aliquot of each subject's urine sample was transferred into five 15-mL clear polypropylene tubes, labeled, and then immediately stored in a cooler with ice packs in the field. The 15-mL tubes were then stored at  $-20^{\circ}\text{C}$  at a local laboratory until shipment to the University of California at Davis for paraquat analysis. Frozen urine samples were airmailed packed in ice on a monthly basis.

### Determination of urinary paraquat

An ELISA was used to determine paraquat concentrations in urine. Briefly, paraquat was extracted from 1 mL of urine using 3 mL Oasis MCX resin cartridges (Waters Corporation, Milford, MA). The individual cartridge could retain up to 100 ng paraquat. The cartridges were eluted with 1 M  $\text{NH}_4\text{Cl}$  in 50% methanol and the elutes were directly determined by ELISA without further additional steps. Extensive quality assurance and quality control were conducted (Koivunen et al. 2005). The average recovery for laboratory fortification of urine samples in concentrations of 5 and 20 ng/mL by ELISA was 91.5% ( $\pm 1.1$ ) for both the concentrations. The correlation between results of laboratory spike paraquat samples from ELISA and liquid chromatography–mass spectrometry was significant ( $R^2 = 0.95$ ). Paraquat in urine samples remained stable under field, storage and transport conditions, which are well reported elsewhere (Koivunen et al. 2005).

### Personal air monitoring

Inhalation exposure to paraquat was measured concurrently with urine collection for a subgroup of the biological monitoring participants. A total of 48 handlers wore inhalable dust samplers during work (daily work hours ranged from 4.5 to 9 h) on paraquat spray days. The sampler consisted of an IOM sampler (SKC Inc, Fullerton, CA) with a 25 mm Teflon filter and an air pump with an airflow rate of 2 L/min (Gilian, GilAir 3, Clearwater, FL). Flow rates were calibrated before and after sampling with an automated bubble low meter (Gilian, Gilibrator, Clearwater, FL). All filters were kept at  $-20^{\circ}\text{C}$  and weighed by a microbalance (Cahn C-35, Madison, WI); each filter was weighed at least thrice and the average weight was used. After weighing the filters, the filter cassettes were stored in tightly sealed Ziploc<sup>®</sup> bags and stored at  $-20^{\circ}\text{C}$  until the filters were analyzed for the concentration of airborne paraquat.

### Determination of airborne paraquat

The extraction procedure was a modification of the method for determining paraquat in soil (Tucker et al. 1967). Each Teflon filter was placed in a plastic 20-mL vial with a 5 mL aliquot of 9 M  $\text{H}_2\text{SO}_4$ . Capped vials were kept horizontally in a shaking water bath at  $60^{\circ}\text{C}$  for 12 h after which each filter was rinsed with 5 mL of deionized water. The rinse was mixed with the extract, and the solution was neutralized with 9 M NaOH. A 4 mL sample of each solution was extracted by vacuum aspiration through a MCX cartridge. After washing the cartridge with 4 mL of 50% (v/v) methanol in water, paraquat was eluted from the cartridge using 4 mL of 1M  $\text{NH}_4\text{Cl}$  in 50% methanol, and the extract was analyzed for paraquat by a UV spectrophotometer

at 256 nm. The concentration of paraquat in the air filter extract was determined using a calibration curve and then verified by a calculation using the extinction coefficient. Air filter extracts were also measured by ELISA. The recovery of radiolabeled paraquat from the filter after a whole process was 96.0% ( $\pm 0.5$ ). The correlation between UV spectrophotometer and ELISA methods to determine paraquat in the air filter was satisfactory ( $R^2 = 0.92$ ). Details are reported elsewhere (Koivunen et al. 2005).

For the UV spectrophotometric method, the LOD was calculated using the formula  $LOD = (3.3 SD)/S$ , where  $S$  is the slope of the standard curve and  $SD$  is the deviation of the y-intercept. The LOQ for paraquat in the air filter extracts was about 0.2  $\mu\text{g/mL}$ , corresponding to 1.3  $\mu\text{g}$  paraquat per filter. UV spectrophotometry was the primary method used for the analysis of paraquat in air filters because the paraquat level in the air filter was so high that the ELISA method required large dilutions of the sample that potentially increased the error on the analysis due to a high sensitivity of ELISA.

### Data analysis

Descriptive statistics were computed for urinary paraquat level. Urinary paraquat level below the LOQ was assigned a value of 1 ng/mL (LOQ/2) for the purpose of statistical analysis. Since the percentage of urine samples below the LOQ was greater than 50%, the range of detectable values was reported in this study. The daily-excreted paraquat is equal to urine concentration multiplied by the daily urine volume. Random effects modeling was used to test for the differences in urinary paraquat across a time period defined as a three-level fixed effect, with spray day as the reference level. An unstructured correlation matrix was used to account for the dependence in observations from urinary paraquat measures in the same handler over time. In analyses where paraquat level was examined as a continuous variable, log transformation was done to give approximately Gaussian distributions. Because some of the biological measurements had small sample sizes, analyses of these variables were primarily descriptive, including univariate (means, standard deviations, frequencies) and bivariate (cross tabs) analyses. Whenever variables had adequate sample sizes, more detailed analyses of association were carried out using analysis of variance, linear regression and random effects modeling. Differences in log-transformed urinary paraquat levels by use of protective equipment were examined using linear regression. The two-tailed Student's  $t$  test was used for two-group comparison.  $P$  values of  $<0.05$  were considered significant in this study. Data were analyzed using SAS, version 8 (SAS Institute, Cary, NC).

### Results

One hundred and nineteen paraquat handlers and 54 non-handlers were recruited, and all participants were male. Coffee farms contributed 64 and 50% of handlers and non-handlers, respectively (Table 1). Non-handlers consisted of 19 workers from 5 banana farms, 27 workers from 10 coffee farms and 8 workers from 1 palm oil farm. The average age and number of years in the current job were similar between handlers and non-handlers. Paraquat spray method was not evaluated in this study because hand-pressurized backpack spraying was the only method at participating farms. Typical work clothing consisted of long pants, long or short sleeved shirts and/or coveralls and boots. Paraquat handlers wore rubber gloves at banana and palm oil farms but not at coffee farms.

The 393 urine specimens collected consisted of 297 samples from 119 paraquat handlers and 96 samples from 54 non-handlers. On the paraquat spray day, a total of 172 urine samples (119 from handlers and 53 from non-handlers) were collected. Ninety-four percentage of handlers ( $N = 112$ ) and 87% of non-handlers ( $N = 46$ ) reported providing complete urine samples. About 94% of urine samples had volumes between 500 and 3,000 mL; nine samples were over 3,000 mL. While 14 samples were less than 500 mL, 11 of these were between 400 and 500 mL.

Mean urine volume was not statistically different for handlers (1,250 mL, SE = 59) and non-handlers (1,210 mL, SE = 88) ( $p = 0.68$ ). Mean urine volume at coffee ( $1,290 \pm 686$  mL), banana ( $1,271 \pm 722$  mL) and palm oil ( $1,168 \pm 485$  mL) farms was not statistically different.

Paraquat was not detected in most of the urine samples from non-handlers. None of the non-handlers had detectable urinary paraquat on days before and after spraying. On the spraying day, 4 out of 53 non-handlers (7.6%) had detectable urinary paraquat. The individual paraquat levels of the four non-handlers were 2.2, 2.9, 4.7 and 6.8  $\mu\text{g}/24$  h.

Urinary paraquat levels for handlers were determined before-, during- and after- paraquat spray days. Due to the spray scheduling and limited access on the weekends, 54 handlers provided urine samples on the day before spraying, and 72 handlers provided samples on the day after spraying. A total of 83.3% ( $N = 45$ ), 47.1% ( $N = 56$ ) and 63.9% ( $N = 46$ ) of the samples were below the LOQ on before, during and after paraquat spray days, respectively (Table 2). Non-detectable urinary paraquat samples were given a half value of LOQ (2 ng/mL). Arithmetic mean of all non-detectable urinary paraquat samples was 1.41 ( $\pm 0.72$ )  $\mu\text{g}/24$  h. Arithmetic means ( $\pm$ SD) and geometric means (GSD) of urinary paraquat levels on spray days were 6.3 ( $\pm 10.45$ ) and 3.0 (3.07)  $\mu\text{g}/24$  h, respectively (Table 2). Arithmetic means ( $\pm$ SD) and geometric means (GSD) of urinary paraquat levels greater than the LOQ samples on spray days were 10.65 ( $\pm 12.90$ ) and 6.42 (2.70)  $\mu\text{g}/24$ h, respectively. Arithmetic means ( $\pm$ SD) of urinary paraquat levels in urinary paraquat samples on before- and afterspray days were 2.03 ( $\pm 1.92$ ) and 2.72 ( $\pm 3.42$ )  $\mu\text{g}/24$ h, respectively. Urinary paraquat levels before the spray day were significantly lower than levels on the spray day using random effects modeling ( $t = -4.96$ ,  $p < 0.001$ ). Urinary paraquat levels were significantly lower after the spray day compared to spray day although this difference was lower than for the before spray day and spray day comparison ( $t = -2.15$ ,  $p = 0.003$ ).

Urinary paraquat levels on spray day were associated with crop in this study (Table 3). The arithmetic means ( $\pm$ SD) of urinary paraquat levels in banana, coffee and palm oil farms were 11.30 ( $\pm 13.48$ ), 5.74 ( $\pm 10.13$ ) and 2.19 ( $\pm 1.94$ )  $\mu\text{g}/24$ h, respectively (Table 3). Detectable paraquat levels were significantly different by crop, with the highest proportion of exposed workers on banana farms—75.0% in banana, 53.9% in coffee and 21.0% for palm oil, ( $\chi^2 = 12.5$ ,  $p = 0.002$ ).

The proportion of workers with detectable levels of paraquat in urine did not vary by the type of job tasks (Table 4). Job tasks included mixing, loading, spraying paraquat and maintaining paraquat-spraying equipment. Because the proportion of workers with detectable urinary paraquat level was significantly different by crop, the association between urinary paraquat exposure and job tasks was stratified on crop (Table 4). Workers in banana and palm oil farms engaged in very similar job tasks. Therefore, it was not possible to make any conclusions regarding associations between job task and urinary paraquat levels. At coffee farms, workers engaged in combinations of paraquat mixing, loading, spraying and equipment maintenance. However, there were no significant differences in the proportion of workers with detectable urinary paraquat levels by individual job tasks.

All workers used boots. Among handlers at coffee, banana and palm oil farms, 66.4% wore a coverall, 38.7% wore gloves, 38.7% used a respirator, and 65.6% wore an apron. Facemasks and safety glasses were rarely used. The use of PPE significantly differed by crop in this study. At banana and palm oil farms, all herbicide handlers used gloves, aprons, respirators and boots when they loaded and sprayed paraquat, and maintained equipment. At coffee farms, use of most types of PPE was low, with the exception of the use of coveralls (48.7%), aprons (48.7%) and boots (100%).

A total of 48 inhalable dust samples were collected from 48 paraquat handlers at nine farms on paraquat spray days, but five dust samples were discarded because the filter papers were damaged. High humidity, rainy conditions and the proximity of the sampler to foliage during sampling were the possible reasons for the filter damage. The arithmetic mean ( $\pm$ SD) and geometric mean (GSD) for inhalable dust exposure for the remaining 43 samples presented in Table 5 were 218.86 ( $\pm$ 253.50) and 112.97 (3.59)  $\mu\text{g}/\text{m}^3$ , respectively.

Eighteen dust samples were accidentally lost during the paraquat extraction process to determine airborne paraquat. The water bath temperature exceeded 100°C during the process due to a broken thermostat, causing the filters to melt. The mean dust level without paraquat analysis ( $N = 18$ ) was slightly but not significantly higher than the level with paraquat analysis ( $N = 25$ ) ( $p = 0.14$ ). The arithmetic mean ( $\pm$ SD) and geometric mean (GSD) for airborne paraquat level measured was 6.07 ( $\pm$  4.77) and 4.75 (2.07)  $\mu\text{g}/\text{m}^3$ , respectively (Table 5). Among 25 handlers with airborne paraquat analysis, 15 (60%) handlers had detectable urinary paraquat level. Airborne and urinary paraquat levels were log-transformed and showed no association in linear regression models ( $\beta = 0.172$ ,  $SE = 0.236$ ,  $R^2 = 0.021$ ,  $F = 0.533$ ,  $p = 0.472$ ).

## Discussion

Field exposure data collection was based on 24-h urine collection. Measurement of paraquat excreted in the urine over 24 h more accurately represents the amount absorbed on a daily basis and has several advantages over spot urine sampling. However, 24-h urine sampling is not an easy task to conduct in field research and subject to both external contamination and incomplete sampling. In addition, 24-h urine sampling requires a sensitive analytical method to measure low concentrations in 24-h urine samples. We applied 24-h urine sampling with careful consideration towards subject convenience, proactive encouragement of complete sampling and availability of a sensitive analytical method. The field staff encouraged participants to collect all voids and report if they missed any samplings. Only 8% of participants reported possibly missing some urine. The completeness of sampling that we achieved may be partially accounted for by providing a convenient carrying bag for the urine containers.

Interpretation of 24-h urine sampling was based on the assumption that urinary paraquat during the sampling day represented exposure for that day. Our data of urinary paraquat levels on before-, during- and after- spray days suggested that the majority of the absorbed paraquat is excreted within 24 h of sampling. Urinary paraquat levels on before and after spray days were significantly lower than on the spray day. A previous animal study showed almost 100% of the absorbed paraquat was eliminated through urine within 24 h (Hawksworth et al. 1981). Since there is no conclusive data on the half-life of paraquat in humans, we applied 24-h urine sampling. However, spot sampling at the end of a shift may be sufficient for future occupational exposure studies. The results of the measurements on spot samples can be corrected for the dilution of the urine (using creatinine concentration or urine gravity). The average urine volume collected on spray day (1,275 mL) was slightly less than the volume reported in other studies; 1,406 mL by 98 professional turf applicators in Canada (Harris et al. 2000) and 1,390 mL by 126 pesticide applicators in Ontario, Canada (Arbuckle et al. 2002). The expected range of 24-h urine volume is 500–3,000 mL based on European population studies (Alessio et al. 1985; Knudman et al. 1986). The location of this study in a different region and climate may in part account for this difference.

It is commonly assumed that agricultural workers are not exposed when they are not handling paraquat. Our biological monitoring data supported this assumption. Urinary paraquat levels clearly indicated a lack of exposure among non-handlers, and exposures were minimal when handlers did not handle paraquat during the workday. When paraquat spraying occurred on the

farms, only 4 out of 53 non-handlers were exposed; however, the level and frequency were negligible. For the few non-handlers with detectable urinary paraquat, it was not possible to identify any determinants of such unanticipated exposure.

The arithmetic mean of urinary paraquat level for handlers on application days was  $6.3 (\pm 10.45) \mu\text{g}/24 \text{ h}$  (range  $<\text{LOQ}-75.37$ ). Several previous exposure studies have measured the urinary paraquat levels of handlers and plantation workers (Chester and Woollen 1981; Staiff et al. 1975; Swan 1969; Van Wendel de Joode et al. 1996; Wojeck et al. 1983). When 24-h urine samples were collected in Sri Lanka, no urinary paraquat was detected using available assay methods with LOD of 30 ng/mL (Chester et al. 1993). Since the previous studies measured paraquat levels with spot urine sampling, we were unable to directly compare values with our results (Chester and Woollen 1981; Staiff et al. 1975; Swan 1969; Van Wendel de Joode et al. 1996; Wojeck et al. 1983). It should be noted that most studies failed to detect paraquat in spot urine samples.

Exposure variations by crop may be explained in part by the length of the workday in the different crops and pesticide handling at participating farms. Work hours for handlers were substantially shorter on the palm oil farm, where we observed lower exposures. Handlers at the palm oil farm worked 5 h per day or less according to farm practice. Handlers at coffee farms worked in the field for about 7 h, and handlers at banana farms worked longer (around 8 or 9 h including time to walk to the field). Paraquat application rates were similar and are not likely to explain observed exposure variations by crop; Gramuron (200 g paraquat ion and 100 g diuron per liter) at the banana farms, Gramoxone Super 20SL (200 g paraquat ion per liter) at the coffee farms and Cafesaquat 20 SL (188 g paraquat dichloride per liter) at the palm oil farm were used. At coffee and banana farms, 1 L paraquat formulation mixed with 200 L water was applied to 1 ha. At the palm oil farm, 2.5 L of paraquat formulation mixed with 87.5 L water was applied to 1 ha. Handlers at coffee and palm oil farms worked as a group, while handlers at banana farms worked individually.

Inhalable dust and airborne paraquat levels were significantly lower than the occupational exposure standard. The highest airborne paraquat level of  $23.8 \mu\text{g}/\text{m}^3$  was significantly less than the ACGIH TLV of  $500 \mu\text{g}/\text{m}^3$  (total dust) (American Conference of Governmental Industrial Hygienists (ACGIH) 2008). The ACGIH TLV of paraquat level in respirable dust is  $100 \mu\text{g}/\text{m}^3$ . The airborne paraquat levels in this study were significantly higher than the levels found in Malaysian plantation workers where average of airborne paraquat levels for sprayers in two different locations were 0.97 and  $0.25 \mu\text{g}/\text{m}^3$  (Chester and Woollen 1981). The difference may be due to shorter spraying times on Malaysian farms and rainy conditions during the sampling. On another study involving Costa Rican banana plantations, the average airborne paraquat level in inhalable dust of eight paraquat applicators was  $1.8 \mu\text{g}/\text{m}^3$  with the range of  $<0.1-24 \mu\text{g}/\text{m}^3$  (Van Wendel de Joode et al. 1996).

Airborne paraquat levels were not associated with urinary paraquat levels in this study. Paraquat has low volatility and droplets containing paraquat may be too large to enter the small airways during application [US Environmental Protection Agency (EPA) 1997]. Paraquat absorption through inhalation may be low because the fraction of respirable particles ( $<5 \mu\text{m}$ ) produced by standard spray nozzles is low. It has been reported that under paraquat-spraying conditions particle sizes appear to be nonrespirable (Swan 1969). Inhalation may not be a major route for occupational paraquat exposure in our study population. Ingestion through oral route may be of importance due to hand-mouth activity. However, lack of association should be interpreted cautiously because of the small sample size for air monitoring data, and no dermal exposure measurements were done in this study.



The findings from our biological monitoring data may not be generalized without careful consideration. This study was conducted in Costa Rica, where administration of pesticide use is relatively well regulated. Participants were recruited from banana, coffee and palm oil farms, covering three major commodities produced in Costa Rica that use paraquat. Since the selection of relatively large farms may not be a representative of all the farms using paraquat, the urinary paraquat level may not represent occupational exposure in Costa Rica. It is possible that these large farms may have more resources for worker health and safety. It is not known if the exposure ranges found in this study are representative of occupational exposure in other countries. Additional exposure assessment studies in other countries may be necessary to compare exposure ranges and identify common determinants of paraquat exposure.

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## References

- Alessio L, Berlin A, Dellorto A, Toffoletto F, Ghezzi I. Reliability of urinary creatinine as a parameter used to adjust values of urinary biological indicators. *Int Arch Occup Environ Health* 1985;55:99–106. doi:10.1007/BF00378371. [PubMed: 3988361]
- American Conference of Governmental Industrial Hygienists (AC-GIH). *Threshold limit values for chemical substances and physical agents and biological exposure indices*; Cincinnati, Ohio. 2008.
- Arbuckle TE, Burnett R, Cole D, Teschke K, Dosemeci M, Bancej C, et al. Predictors of herbicide exposure in farm applicators. *Int Arch Occup Environ Health* 2002;75:406–414. doi:10.1007/s00420-002-0323-7. [PubMed: 12070637]
- Blake DK, Gallagher RT, Woollen BH. Improved methods for the analysis of paraquat in biological fluids. *Chromatographia* 2002;55:183–185. doi:10.1007/BF02493377.
- Chester G, Woollen BH. Studies of the occupational exposure of Malaysian plantation workers to paraquat. *Br J Ind Med* 1981;38:23–33.
- Chester G, Gurunathan G, Jones N, Woollen BH. Occupational exposure of Sri Lankan tea plantation workers to paraquat. *Bull World Health Organ* 1993;71:625–632. [PubMed: 8261566]
- Daniel JW, Gage JC. Absorption and excretion of diquat and paraquat in rats. *Br J Ind Med* 1966;23:133–136. [PubMed: 5929687]
- Hajslova J, Cuhra P, Davidek T, Dacidek J. Gas chromatographic determination of diquat and paraquat in crops. *J Chromatogr A* 1989;479:243–250. doi:10.1016/S0021-9673(01)83340-9.
- Harris SA, Purdham JT, Corey PN, Sass-Kortsak AM. An evaluation of 24-hour urinary creatinine excretion for use in identification of incomplete urine collections and adjustment of absorbed dose of pesticides. *Am Ind Hyg Assoc J* 2000;61:649–657. doi 10.1202/0002-8894(2000)061<0649:AEOUCE>2.0.CO;2.
- Hawksworth GM, Bennett PN, Davies DS. Kinetics of paraquat elimination in the dog. *Toxicol Appl Pharmacol* 1981;57:139–145. doi:10.1016/0041-008X(81)90273-8. [PubMed: 7222029]
- Knuiman JT, Hautvast JGA, Van der Heyden L, Geboers J, Joossens JV, Tornqvist H, et al. A multi-centre study on completeness of urine collection in 11 European centres. I. Some problems with the use of creatinine and 4-aminobenzoic acid as markers of the completeness of collection. *Hum Nutr Clin Nutr* 1986;40:229–237. [PubMed: 3487532]

- Koivunen M, Gee SJ, Park E-K, Lee K, Schenker MB, Hammock BD. Application of an enzyme-linked immunosorbent assay for the analysis of paraquat in human-exposure samples. *Arch Environ Contam Toxicol* 2005;48:184–190. doi:10.1007/s00244-003-0251-x. [PubMed: 15696345]
- Staiff DC, Comer SW, Armstrong JF, Wolfe HR. Exposure to the herbicide, paraquat. *Bull Environ Contam Toxicol* 1975;14:334–340. doi:10.1007/BF01685647. [PubMed: 1174749]
- Swan AAB. Exposure of spray operators to paraquat. *Br J Ind Med* 1969;26:322–329. [PubMed: 5346830]
- Tompsett SL. Paraquat poisoning. *Acta Pharmacol Toxicol (Copenh)* 1970;28:346–358. [PubMed: 5536735]
- Tucker BV, Pack DE, Ospenson JN. Adsorption of bipyridylum herbicides in soil. *J Agric Food Chem* 1967;15:1005–1008. doi:10.1021/jf60154a017.
- U.S. Environmental Protection Agency (EPA). Reregistration eligibility decision (RED), paraquat dichloride. Office of Prevention, Pesticides and Toxic Substances (EPA 738-F-96-018); 1997.
- Van Emon J, Hammock B, Seiber JN. Enzyme-linked immunosorbent assay for paraquat and its application to exposure analysis. *Anal Chem* 1986;58:1866–1873. doi:10.1021/ac00121a057. [PubMed: 3752512]
- Van Wendel de Joode B, De Graaf IAM, Wesseling C, Kromhout H. Paraquat exposure of knapsack spray operators on banana plantations in Costa Rica. *Int J Occup Environ Health* 1996;2:294–304. [PubMed: 9933884]
- Wojeck GA, Price JF, Nigg HN, Stamper JH. Worker exposure to paraquat and diquat. *Arch Environ Contam Toxicol* 1983;12:65–70. doi:10.1007/BF01055003. [PubMed: 6830311]

**Table 1**

Demographic characteristics of study participants

	<b>Handler (n = 119)</b>	<b>Non-handler (n = 54)</b>
	<i>N (%)</i>	<i>N (%)</i>
Crop		
Banana	24 (20.2%)	19 (35.2%)
Coffee	76 (63.9%)	27 (50.0%)
Palm oil	19 (16.0%)	8 (14.8%)
Age (year) (mean ± SD)	36.8 ± 10.7	35.7 ± 12.3
Working years in current job* (mean ± SD)	8.8 ± 8.2	8.0 ± 8.4

\* Current work history of less than 6 months was coded as 0 years, but 6–12 months was coded as 1 year

**Table 2**

Urinary paraquat levels from paraquat handlers on before-, during- and after- spray days

	<b>Before (N = 54)</b> <b>(µg/24 h)</b>	<b>During (N =119)</b> <b>(µg/24 h)</b>	<b>After (N = 72)</b> <b>(µg/24 h)</b>
Arithmetic means (± SD)	2.03 (±1.92)	6.3 (±10.45)	2.72 (±3.42)
Geometric means (GSD)	1.58 (1.90)	3.00 (3.07)	1.82 (2.23)
N (%) <LOQ	45 (83.3%)	56 (47.1%)	46 (63.9%)
Range	<LOQ–10.46	<LOQ–75.37	<LOQ–19.90

Samples less than the LOQ (2 ng/mL) are given a half value

**Table 3**

Urinary paraquat levels for handlers on spray day by crop

	<b>Coffee (N =76)</b> <b>(µg/24 h)</b>	<b>Banana (N = 24)</b> <b>(µg/24 h)</b>	<b>Palm oil (N =19)</b> <b>(µg/24 h)</b>
Arithmetic means (±SD)	5.74 (±10.13)	11.39 (±13.48)	2.19 (±1.94)
Geometric means (GSD)	2.91 (2.87)	5.22 (3.88)	1.67 (2.02)
N (%) <LOQ	35 (46.1%)	6 (25.0%)	15 (79.0%)
Range	<LOQ–75.37	<LOQ–54.21	<LOQ–7.25

Samples less than the LOQ (2 ng/mL) are given a half value

**Table 4**

Comparison of detectable urinary paraquat by job tasks on spray day

Job tasks	Detected urinary paraquat (%)		
	Coffee farms (N = 76)	Banana farms (N = 24)	Palm oil farm (N = 19)
Mixing			
Yes	53.9 (14/26)	0 (0/0)	0 (0/2)
No	54.0 (27/50)	75.0 (18/24)	23.5 (4/17)
Loading			
Yes	55.1 (38/69)	75.0 (18/24)	23.5 (4/17)
No	42.9 (3/7)	0 (0/0)	0 (0/2)
Spraying			
Yes	54.9 (39/71)	75.0 (18/24)	23.5 (4/17)
No	40.0 (2/5)	0 (0/0)	0 (0/2)
Maintaining equipment			
Yes	36.4 (4/11)	100 (8/8)	23.5 (4/17)
No	56.9 (37/65)	62.5 (10/16)	0 (0/2)

**Table 5**

Inhalable dust and airborne paraquat levels on spray day

Handlers (N =43)	Arithmetic mean ( $\pm$ SD)	Geometric mean (GSD)	Range
Inhalable dust ( $\mu\text{g}/\text{m}^3$ )	218.86 ( $\pm$ 253.50)	112.97 (3.59)	2.30–1032.20
Airborne paraquat ( $\mu\text{g}/\text{m}^3$ )*	6.07 ( $\pm$ 4.77)	4.75 (2.07)	1.00–23.80

\* A total of 25 samples due to accidental loss of 18 samples during the analysis process